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Sterility Evaluation After Pre-Use Integrity Test of Feed Side Contaminated Filters by Bacteria Challenge Test

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Abstract

Sartorius provides the most advanced sterile filters for various filtration applications in the biopharmaceutical industry. Sartorius offers a broad variety of filters with different pore size combinations, which ensures highly economical, user-friendly, and safe filtration for an industry that is increasingly using pre-qualified single-use systems, such as filter transfer sets.

Several regulatory guidelines recommend pre-use integrity testing (IT) of critical sterilizing liquid filters for aseptic processing. Pre-use, post-sterilization integrity testing (PUPSIT) is performed after single-use systems or filters are installed to ensure that the sterilization and installation processes have not damaged the filter prior to product filtration. Many customers are concerned about system sterility when a pre-use IT is performed. The question arises of whether the pressure applied during the pre-use IT or the post-use product recovery test will push the bacteria through the filter and compromise the sterility of the system. To demonstrate the bacterial retention ability of our filters, a bacteria challenge test (BCT) is performed after a wetting contaminated with bacteria. This is followed by pre-use IT for standard sterilizing grade filters used in Sartorius filter transfer sets.

Introduction

Bacteria Challenge Test for Feed Side Contaminated Filters After Integrity Test

Sterilizing-grade filters are used widely in sterile drug manufacturing, and it is mandatory that any drug product is passed through an integral filter (failing to do so poses a high risk to patients). Performance of a pre-use, post-sterilization integrity test (PUPSIT) is required by Section 113 of Annex 1 of Volume 4 of the EU GMPs and applies to the filtration of sterile medicinal products that cannot be sterilized in their final container. Section 113 states, "The integrity of the sterilized filter should be verified before use." As written, the guidance indicates that the integrity of the filters in question should be determined after sterilization but before use.

A PUPSIT tests the integrity of the sterilizing filter and assembly, including the filter housing, support structure, and connection after it has been installed and sterilized and before it is used to filter product[2]. It is performed once a sterilizing filter has been installed to ensure the sterilization and installation process has not damaged the filter prior to product filtration. The integrity test (IT) is usually performed using a bubble point, diffusive flow, or pressure hold test.

Many drug manufacturers consider it difficult to perform a PUPSIT without breaching system sterility[3]. This begs the question: will the pressure applied during the IT push the possible contaminant present in the wetting media to the filtrate side of the system? To demonstrate the bacterial retention ability of our filters used in the filter transfer set final filling assemblies (Figure 1), a bacteria challenge test (BCT) is performed after a wetting contaminated with bacteria. This is followed by a pre-use IT for standard sterilizing grade filters used in filter transfer set final filling assemblies. The test filters are provided in Table 1.

In this study, the test filters were wetted with contaminated water (i.e., water containing *Brevundimonas diminuta* in the concentration of 10^7 CFU/cm²), followed by an IT using the Sartocheck®. Soon afterward, a BCT was performed without removing the filters from the test setup to check if the pressure applied during the integrity test pushed the bacteria to the filtrate side[1]. The BCT was performed in accordance with ASTM 838-15a – "Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration." Both tests were performed with two filters from each filter type in the BCT rig to mimic a closed system.

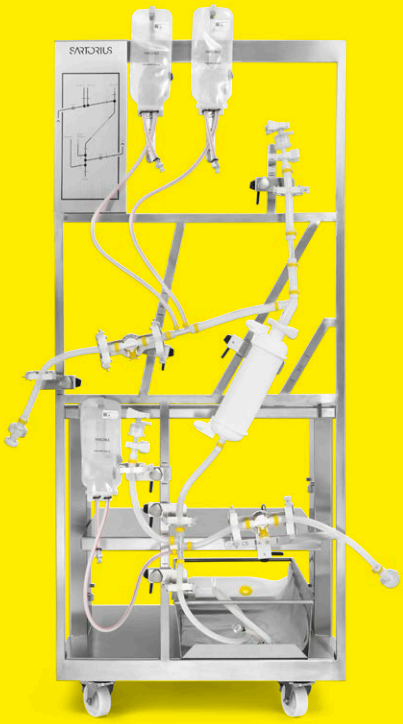

Figure 1: Stainless steel holder with filter transfer set final filling assembly.

Filter Type	Order Number	Membrane Material	Treatment	Pore Size	Filtration Area
Sartobran® P	5235307H9-SS	Cellulose acetate (CA)	Autoclaved	0.2 µm	0.2 m²
Sartopore® 2	5445307H9G-SS	Polyethersulfone (PES)	Gamma irradiated	0.2 µm	0.2 m²
Sartopore® 2 XLG	5445307G9G-SS	Polyethersulfone (PES)	Gamma irradiated	0.2 µm	0.26 m²
Sartopore® 2 XLI	5445307I9G-SS	Polyethersulfone (PES)	Gamma irradiated	0.2 µm	0.26 m²
Sartopore® Platinum	5495307H9G-SS	Modified Polyethersulfone (PES)	Gamma irradiated	0.2 µm	0.26 m²
Sartopore® 2 XLM	5445358M9G-SS	Polyethersulfone (PES)	Gamma irradiated	0.1 µm	0.26 m²

Table 1: Filters used in filter transfer set final filling.

Experiment Flow

Filter Wetting

- Connect the test filters to the rig
- Filter wetting as per standard wetting procedure.

Integrity Test

- Test diffusion & bubble point, without removing the filters from the test setup.

Contaminated Water Wetting

- Pass contaminated water containing 1×10^7 cfu/cm² of *B. diminuta* culture (BCT)
- Perform growth test for the challenge solution to confirm the challenge level

Integrity Test

- Test diffusion & bubble point, without removing the filters from the test setup

BCT

- Perform BCT by challenging 1×10^7 cfu/cm²
- Perform growth test for the challenge solution to confirm the challenge level

Integrity Test

- Test diffusion & bubble point

Incubation

- Transfer Analytical filters aseptically to the growth media (TSA)
- Incubate analytical filter and growth test plates of challenge solutions at 30 °C for 5 days.

Result

- Compare the Integrity test results of before and after test.
- After incubation period, check for colonies in analytical filters – sterile results indicate test pass

Results – Integrity Test

Diffusion and bubble point test results indicate the structural integrity of the test filters, IT test values before contamination, after contamination, and after the BCT were compared and are provided in the table below (average of two tests performed for each filter type). IT values were within acceptance range of each test filter type, indicating test filters were intact after two BCT and IT tests.

Filter Name	Diffusion (mL/min)				Bubble Point (bar)			
	Maximum Limit	Before Test	After Contamination	After BCT	Minimum Limit	Before Test	After Contamination	After BCT
Sartobran® P	5	2.0	2.2	3.5	3.2	3.72	3.75	3.74
Sartopore® 2	7	5.4	3.5	4.2	3.2	3.39	3.77	3.49
Sartopore® 2 XLG	9	6.9	4.7	5.6	3.2	3.63	3.55	3.55
Sartopore® 2 XLI	8	4.4	4.5	4.3	3.2	4.05	4.15	3.93
Sartopore® Platinum	7	3.9	3.8	3.9	3.5	3.72	3.87	3.82
Sartopore® 2 XLM	12	8.5	7.8	7.8	NA	NA	NA	NA

Table 2: Diffusion and bubble point values of the test filters before contamination, after contaminated wetting, and after BCT.

Results – Bacteria Challenge Test

The results of the BCT were analyzed by incubating the analytical filter at 30 °C for five to seven days. Sterile results were observed – the analytical filters were without any colony forming units (CFU) in all the filters tested, which indicates that the filters retained the challenge bacteria during the test. Examples of the analytical filters after incubation without any CFUs are shown in Figure 2.

To ensure the correct concentration of bacteria in the challenge solution, a growth promotion test of challenge solution was performed. A challenge concentration of $>1 \times 10^7$ CFU/cm² was achieved in our experiment.
The results of the test challenge concentration are shown in Figure 3.

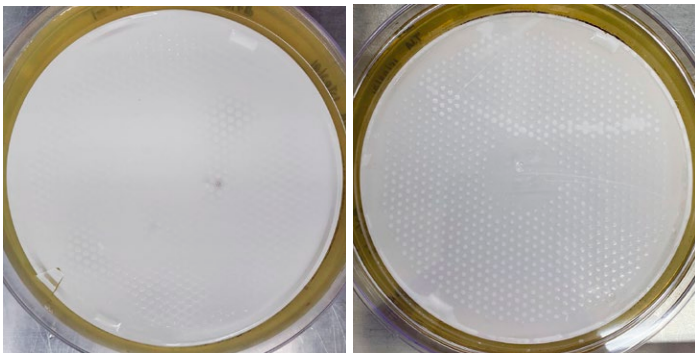

Figure 2: Examples of sterile results on analytical filters after two BCTs.

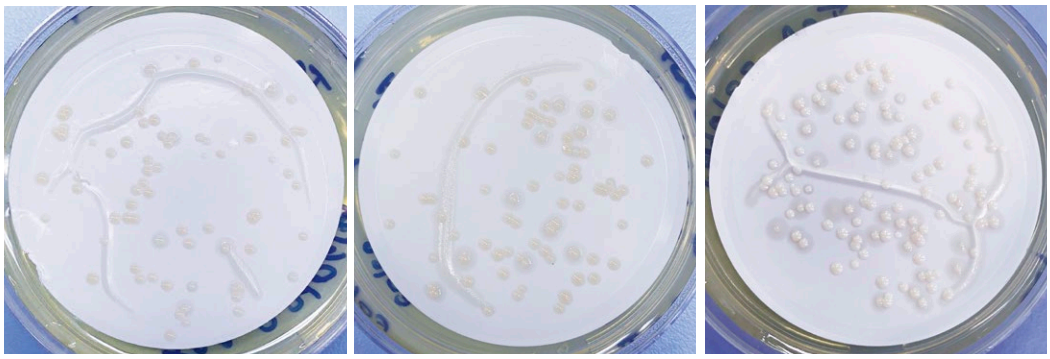

Figure 3: *Brevundimonas diminuta* in the challenge solution.

Bacterial challenge results are also expressed in terms of log reduction value (LRV) as a description for the microbial removal efficiency of a filter. A filter with an LRV of seven can reduce the organisms in the feed stream by seven orders of magnitude.

The BCT result, including the challenge concentration, colonies on the analytical filter, and LRV values, are depicted in Table 3.

Filter Name	Challenge by Test Solution ($\times 10^7$ CFU/cm^2)	Colonies on Analytical Filter	LRV	Test Result
Sartobran® P	1.13	0	7.1	Pass
Sartopore® 2	1.30	0	7.1	Pass
Sartopore® 2 XLG	1.26	0	7.0	Pass
Sartopore® 2 XLI	1.46	0	7.2	Pass
Sartopore® Platinum	1.54	0	7.2	Pass
Sartopore® 2 XLM	1.56	0	7.2	Pass

Table 3: Bacteria challenge test result.

Conclusion

The data presented demonstrates that the pressure applied during the integrity test after a highly contaminated water wetting does not cause bacterial breakthrough into the filtrate side.

The microbial retention capacity of the Sartorius filters ensures the sterility of the products. A pre-use IT test, or product recovery using compressed air pressure up to the bubble point of Sartorius filters or filter transfer sets, will mitigate the risks associated with shipment and installation without compromising the sterility and quality of the products.

References

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